REMARKS/ARGUMENTS

By the present amendment, claims 1, 2, 10, 11, 13, 19, 22, 23, 27, 30 and 34 have been amended as described below and claims 20, 21, 25, 26 and 32 have been cancelled. The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further divisional, continuation or continuation-in-part application. No new matter has been entered by the present amendment and its entry is respectfully requested.

The Official Action dated February 28, 2005 has been carefully considered. It is believed that the amended specification and claims and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Specification

The Examiner has objected to the specification as it contains embedded hyperlinks. In response, page 21 has been amended in order to delete reference to the hyperlinks at pages 22 and 26.

Sequence Compliance

We do not understand the objection to the application as failing to provide Sequence Identifier Numbers, for example, on page 20, line 13 and Table 12. In the amendment that was filed on September 12, 2003, the application was amended in order to include the Sequence Identifier Numbers. A copy of that amendment is enclosed for the Examiners reference.

Claim Objections

The Examiner has objected to Claim 13 as the word "transgene" was misspelled on line 2. In response, the typographical error has been corrected.

35 USC §112, First Paragraph

The Examiner has objected to claims 1-35 under 35 USC §112, first paragraph, as lacking enablement. We note that the Examiner does confirm the enablement of a transgenic non-human mammal whose genome comprises a transgene comprising a gene encoding a phytase operably linked to a mammalian salivary gland specific promoter wherein the animal is a pig, goat, sheep, cow, or horse, wherein the phytase is expressed in the salivary gland of the animal. We respectfully submit that the specification does enable the full scope of claim for the reasons that follow. We will address the three specific points that the Examiner raises on pages 9-11 of the office action in the order in which they appear.

1) The Examiner alleges that the specification is not enabled for the preparation of poultry or fish. In response, claims 1, 13 and 19 have been amended in order to delete reference to fish and poultry in order to expedite prosecution.

2) The Examiner is of the opinion that the specification is not enabling for using the transgenic non-human animal wherein expression of the transgene does not occur or occurs anywhere either than the salivary glands. In response, claims 1 and 13 have been amended in order to specify that the protein is expressed. However, we respectfully submit that the specification does enable the expression of the transgene in tissues other than the salivary glands. In this regard, we are enclosing a copy of an article by Golovan et. al. (Nature Biotechnology, 2001, volume 19, pp. 741-745) which is the publication of the inventors' work in a highly reputable scientific journal. As can be seen by Table 2 of the Golovan et. al. reference, the inventors have shown that the phytase can be produced in other tissues such as the fundus region of the stomach and

the duodenum. The inventors also have unpublished data that shows that the phytase is also expressed in the upper alimentary tract, respiratory tract mucosa and the reproductive tract. As a result, the specification is enabling for the production of the protein in other tissues.

3) The Examiner is of the opinion that the specification is not enabling for a transgene encoding any protein or any glycoprotein. We respectfully disagree with the Examiner. The inventors were the first to prepare a large transgenic animal expressing a protein under the control of a salivary gland specific promoter. Once the inventors had demonstrated that it could be done by creating a transgenic pig expressing phytase, one of skill in the art could readily prepare transgenic animals expressing other proteins. The specification provides sufficient methodology to allow one of skill in the art to carry out the full scope of the claims without undue experimentation.

Notwithstanding the above, the claims have been amended to specify that the transgene encodes a phytase in order to expedite prosecution of the application. This amendment is without prejudice to applicant pursuing the broader scope of claim in a further application.

In view of the foregoing, we respectfully submit that all of the objections to the claims under 35 USC§112, first paragraph be withdrawn.

35 USC§103

The Examiner has objected to claims 1-5, 8, 9, 19-25, 29-32 and 35 under 35 USC§103(a) as being unpatentable over Mikkelsen (1992, Nucl. Acids Res., Vol. 20, pages 2249-2255) in view of Velander (1992, PNAS, Vol. 89, pages 12003-12007). We respectfully disagree with the Examiner for the reasons that follow.

Mikkelsen relates to transgenic mice comprising a transgene encoding human factor VIII operably linked to a salivary-specific Lama PSP regulatory region. The claims of the

present application are directed to the preparation of larger transgenic animals such as pigs, goats, sheep, cows and horses. We respectfully submit that the murine data presented in Mikkelsen would in no way lead one of skill in the art to the present invention ***with a reasonable expectation of success***. The preparation of larger transgenic animals would in no way be expected by one of skill in the art having read Mikkelsen. In fact, when the inventors published their initial paper on the production of transgenic mice expressing a phytase (Nature Biotechnology. 2001. Vol. 19, p. 429-433), a review of their work was published in the same volume by Kevin Ward who is a senior principal research scientist at CSIRO Animal Production in Australia. We enclose a copy of Dr. Ward's commentary. We direct the Examiner to the following passage:

> "The next step in this line of research is clearly to repeat these studies in a farm animal, such as the pig, one of the prime industrial targets."

The paper goes on to state that:

> "It is not unknown for transgenes to function as predicted in transgenic mice but not in larger animals, and as a consequence, the suitability of the laboratory mouse as a model for transgene expression studies in larger animals is unclear. Furthermore, previous attempts to alter the digestive capabilities of larger animals by enzyme-encoding transgenes have not succeeded, despite their promise in laboratory mice."

We submit that one of skill in the art would therefore be unable to predict that the preparation of a transgenic animal expressing a protein in the saliva or gastrointestinal tract would work especially when the animal is a larger, more complicated one, such as a pig. In further support of this, we draw the Examiner's attention to an article by Mullaney et al. (Advances in Phytase Research found in Advances in Applied Microbiology, Vol. 47, 2000) wherein it is stated on page 185 that:

> "In the future, transgenic poultry, hogs, and so on may produce phytase in their own digestive tract. Several attempts have already been made to transform and express a fungal phytase in an animal (privileged information, personal communication). To date none of these attempts have been successful Similar results were obtained when the *phy*A gene was expressed in *E. coli* (Phillippy and Mullaney, 1997)."

We are enclosing the relevant passage from this text.

In addition to the above remarks by experts in the art, at the time of the invention there were numerous examples in the scientific literature where the production of larger transgenic animals has failed. In particular, scientists have attempted over expression of growth hormone (Pursel et al., Science 244:1281-1287, 1989) and IGF-1 (Pursel et al., *In* Transgenic animals in agriculture. Edited by J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin. CABI Publishing. New York. pp. 131-144, 1999; Ward, K.A., In Ruminant Physiology Digestion, Metabolism, Growth and Reproduction. Edited by P.B. Cronjé. CABI Publishing. Wallingford. pp. 373-387, 2000a) to enhance the growth or carcass characteristics of pigs, introduction of the glyoxylate pathway into sheep (Ward, K.A., Trends Biotechnol. 18:99-102, 2000b), and enhanced cysteine biosynthesis in sheep (Ward, K.A., Trends Biotechnol. 18:99-102, 2000b) for other beneficial purposes. In the case of growth hormone (Pursel et al., 1989), glyoxylate pathway (Saini et al., Transgenic Res. 5: 467-473, 1996) and the cysteine biosynthesis pathway (Bawden et al., Transgenic Res. 4: 87-104, 1995), expression of the transgenes in mice produced the expected results. However, in the large domestic animals these transgenes were either not expressed or led to deleterious secondary effects. Furthermore, as mentioned above, in a recent review Mullaney et al. (Advances in phytase research. Adv. Appl. Microbiol. 47: 157-199, 2000) it was reported that several attempts have been made to transform and express a fungal phytase in an animal without success. Therefore without demonstrating transgene function in the large domestic animal, reliable predictions of outcome cannot be made (see review by V.G. Pursel, In Animal breeding: Technology for the 21st century. Edited by A.J.Clark. Harwood Academic Press. Amsterdam. pp. 183-200, 1998) and the critique by K.A. Ward (which is enclosed), which bare out this statement. It is also worth noting that not only have the inventors successfully (and unexpectedly) prepared a transgenic animal expressing phytase, they have shown the phytase is functional and is effective at lowering fecal phosphorus. The invention clearly has merit and has solved an unmet need in the art.

The deficiencies in Mikkelsen are in no way remedied by Velander which teaches a transgenic pig expressing human protein C. Velander uses a mouse whey acidic promoter and not a salivary gland specific promoter as taught in the present claims. Further, Velander is not concerned with the expression of phytase which is required by the amended claims.

The Examiner has objected to claims 1, 10, 11, 13-17, 19, 26, 27, 30 and 34 under USC§103(a) as being unpatentable over Mikkelsen, (1992, Nucl. Acids Res., Vol. 20, pages 2249-2255) in view of Velander (1992, PNAS, Vol. 89, pages 12003-12007), and further in view of Pen et. al. (1993, Bio/technology, Vol. 11, pages 811-814) as evidenced by Laursen (1997, Gene, Vol. 198, pages 367-372). We respectfully disagree with the Examiner for the reasons that follow.

Our comments on Mikkelsen on Velander appear above. Pen et. al. is concerned with transgenic seeds expressing phytase. One of skill in the art would not expect that data in transgenic plants would be predictive of the ability to prepare a large transgenic animal. Further, Pen et. al. provides no motivation to one of skill in the art to attempt to prepare a transgenic pig as it teaches that pigs can eat the transgenic seeds as a means of reducing phosphorous excretion. Laursen et. al. relates to the expression of human factor VIII in the salivary glands of mice. As mentioned above in our discussion of Mikkelsen et. al., data in mice is not predictive of the outcome in larger animals.

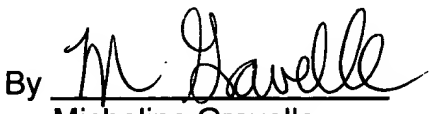
In view of the foregoing, we respectfully submit that all of the objections to the claims under 35 USC§103 be withdrawn.

The Commissioner is hereby authorized to charge any fee (including any claim fee) which may be required to our Deposit Account No. 02-2095.

In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance and early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, she is kindly requested to contact the undersigned by telephone at (416) 957-1682 at her convenience.

Respectfully submitted,

BERESKIN & PARR

By 
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Attachments

THE PATENT AND TRADEMARK OFFICE ACKNOWLEDGES AND HAS STAMPED HEREON THE DATE OF RECEIPT OF THE ITEMS CHECKED BELOW:

DATE: September 12 200

Applicant: Carol W Forsberg et al. File No. 6580-270

Title of Invention Transgenic Animals Expressing Salivary Proteins

Serial No. 09/926,375 Filing Date: Febuar. 28 2

Patent No.: Due Date: Septem 13 20

Method of Payment Amount:

APPLICATION PAPERS

- ☐ Abstract
- ☐ Application Data Sheet
- ☐ Claims (No. of Claims)
- ☐ Continued Prosecution Application (CPA) Request Transmittal
- ☐ Fee Transmittal
- ☐ Formal/ Informal Drawings (Sheets)
- ☐ New Design Patent Appln. Transmittal
- ☐ New Plant Patent Appln. Transmittal
- ☐ New Utility Patent Appln. Transmittal
- ☐ Provisional Appln. for Patent Cover Sheet
- ☐ Specification (No. of Pages)
- ☐ Transmittal Concerning Filing Under 35 U.S.C. 371

OTHER PAPERS

- ☐ Assignment
- ☐ Declaration/Oath
- ☐ Information Disclosue Statement
- ☐ Issue Fee NLA
- ☐ Letter
- ☐ Maintenance Fee Transmittal Form
- ☒ Preliminary Amendment
- ☐ Priority Document
- ☐ Request for Ext. of Time
- ☐ Resp. to Office Action Dated August 13 200
- ☐ Transmittal Form
- ☒ Sequence Listing / Diskette
- ☐

STAMP HERE

Appl. No	:	09/926,375 Confirmation No.: 9974
Applicants	:	Cecil W. Forsberg et al.
Filed	:	February 28, 2002
Title	:	Transgenic Animals Expressing Salivary Proteins
TC./A.U.	:	1632
Examiner	:	Valarie E. Bertoglio
Docket No.	:	6580-270
Customer No.	:	001059

Honorable Commissioner for Patents
P. O. Box 1450
Alexandria, Virginia 22313–1450

RESPONSE

Sir:

The present letter is filed in response to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures dated August 13, 2003.

Amendments to the Specification begin at page 2 of this paper

Amendments to the Claims begins on page 6 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Amendment to the Specification

Please replace Sequence Listing pages 57-87 currently on file with the enclosed Sequence Listing pages 57-103.

Please replace the paragraph beginning at page 4, line 23, with the following amended paragraph:

--Figure 1 is a schematic diagram representing a method for producing the gene construct of the present invention containing the inducible proline-rich protein (PRP) promoter/enhancer. More specifically, Figure 1 is a schematic diagram illustrating the steps in the construction of the transgenes R15/APPA+intron and R15/APPA used for the generation of transgenic mice. (SEQ ID NO: 36)--

Please replace the paragraph beginning at page 4, line 32, with the following amended paragraph:

--Figure 3 is a schematic diagram representing a method for producing the gene construct of the present invention containing the constitutive parotid secretory protein (PSP) promoter/enhancer. More specifically, Figure 3 is a schematic diagram illustrating the steps in construction of the transgenes Lama2/APPA that codes for the native AppA phytase and the Lama2/PSP/APPA that codes for the AppA phytase with the PSP signal peptide sequence. (SEQ ID NOS: 37 & 38)--

Please replace the paragraph beginning at page 19, line 9, with the following amended paragraph:

--Because a large part of Lama 2 had not been sequenced, the construct was first disassembled and subcloned into pBluescript KS(+) and after incorporation of the *APPA* gene, the Lama 2 was reassembled back (Figure 3). We used unique enzymes RsrII and Sma1 to remove a 3.4 kbp fragment from Lama2, which was subcloned into

the multiple cloning site (MCS) of pBluescript II KS(+) that was previously digested with Kpn1 and Sma1, using a Kpn1-RsrII adapter (Figure 3, step 1).

KpnI* RsrII

TGGGAGGTCG (SEQ ID NO: 8)

CATGACCCTCCAGCCAG (SEQ ID NO: 9)--

Please replace the paragraph beginning at page 19, line 18, with the following amended paragraph:

--That allowed us to preserve the RsrII (CG/GWCCG) (SEQ ID NO: 10) site and destroy the Kpn1 site (GGTAC/C (SEQ ID NO: 11)> GGTAC/T (SEQ ID NO: 12)), which would otherwise interfere with future cloning. The pKS/Lama construct was digested with Apa1 and Kpn1 and used in a three-way ligation with the modified APPA (Figure 3, step 2). We designed two PSP/APPA constructs. One construct APPA-signal/APPA (Figure 3, steps 3a-7a) had the original bacterial signal sequence from the APPA protein having the following amino acid sequence:

Met-Lys-Ala-Ile-Leu-Ile-Pro-Phe-Leu-Ser-Leu-Leu-Ile-Pro-Leu-Thr-Pro-Gln-Ser-Ala-Phe-Ala (SEQ ID NO: 13)--

Please replace the paragraph beginning at page 19, line 28, with the following amended paragraph:

--We also modified a sequence near the ATG codon to resemble the optimal mammalian Kozak sequence (GCC GCC A/GCC ATG G) (SEQ ID NO: 14) (Kozak 1987), but we did not mutagenize the +4 position because it would change Lys to Glu in the signal sequence with possible deleterious consequences for protein export. This optimized sequence was used in our previous construct R15/APPA and led to high levels of phytase production. We checked the APPA bacterial signal sequence using the PSORT computer neural network trained on eukaryotic signal sequences and further described at http://psort.nibb.ac.jp:8800/ (Nakai and Kanehisa 1992). The APPA

bacterial signal sequence was recognized as an efficient leader peptide and the cleavage site was correctly predicted. PSORT also predicted that there is a high probability that phytase would be exported correctly outside of the cell. There were also publications showing that some bacterial signal sequences might function efficiently in mammalian cells (Williamson *et al.* 1994) (Hall *et al.* 1990). Our experiments using cell culture demonstrated that the APPA signal was correctly processed with export of phytase outside of the cell.--

Please replace the paragraph beginning at page 20, line 8, with the following amended paragraph:

--Experiments using cell culture cannot predict the direction of export and if phytase were exported into blood vessels instead of salivary ducts that could lead to deleterious effects. That is why we also designed a second construct PSP-signal/APPA (Figure 3, steps 3b-7b) that would preserve the original PSP signal amino acid sequence:

Met-Phe-Gln-Leu-Gly-Ser-Leu-Val-Val-Leu-Cys-Gly-Leu-Leu-Ile-Gly-Asn-Ser-Glu-Ser (SEQ ID NO: 15).--

Please replace the Table 12 beginning at page 47, with the following amended Table 12:

--Table 12. Primers used for construction and detection of transgenic constructs.

Name	Start-End[1]	Forward/ Reverse	
Primers used in R15/APPA+intron and R15/APPA construction			
APPA-DOWN2		R	TCGGCGCTCACCTTGAGTTC (SEQ ID NO: 16)
APPA-DRA		F	CCGTTTAAAGCCATCTTAATCCCAT (SEQ ID NO: 17)
APPA-SMA		R	GTCCCGGGTATGCGTGCTTCATTC (SEQ ID NO: 18)
CAT-ATG		R	CCATGGTGGCGGCTTTTAGCTTCCTTAGCTCCTGA (SEQ ID NO: 19)
CAT-TAA		F	AGCGCTTGCAGTTTGTAAGGCAGTTATTGGTGCCC (SEQ ID NO: 20)

CAT-UP1		F	TCG AGG AGC TTG GCG AGA TT (SEQ ID NO: 21)
R15-UP1		F	TTTCGGGCCAATGTTGCTGT (SEQ ID NO: 22)
Primers used in SV40/APPA+intron construction			
SV-HIND		F	CCCAAGCTTTACACTTTATGC (SEQ ID NO: 23)
SV-XHO		R	GCCCTCGAGCCTCCTCACTACTTCT (SEQ ID NO: 24)
Primers used in Lama2/APPA and Lama2/PSP/APPA construction			
APPA-CLA	12635-12657	F	GGATCGATAAAAGCCGCCACCATGAA (SEQ ID NO: 25)
APPA-DOWN2	13307-13326	R	TCGGCGCTCACCTTGAGTTC (SEQ ID NO: 26)
APPA-DOWN4	12751-12780	R	GCACGCACACCATGACGACTGACAATCACC (SEQ ID NO: 27)
APPA-KPN	13935-13959	R	CGGGTACCTTACAAACTGCAAGCGG (SEQ ID NO: 28)
APPA-MATURE	12719-12738	F	CAGAGTGAGCCGGAGCTGAA (SEQ ID NO: 29)
APPA-UP2	13210-13229	F	CGAACTGGAACGGGTGCTTA (SEQ ID NO: 30)
LAMA-CLA	12615-12639	R	GCATCGATCTTTGGTTCTGACAAATGG (SEQ ID NO: 31)
LAMA-SIGNAL		R	TGACTCTGAGTTCCCAATGA (SEQ ID NO: 32)
LAMA-UP	12111-12130	F	GTGCTGCTCCAAGTTTGGTG (SEQ ID NO: 33)
Primers for detection of the porcine β-globin gene			
PIG-BGF		F	GCAGATTCCCAAACCTTCGCAGAG (SEQ ID NO: 34)
PIG-BGR		R	TCTGCCCAAGTCCTAAATGTGCGT (SEQ ID NO: 35)

1 The location of the primers shown for Lama2/APPA sequence.
The start and stop codons of *APPA* are indicated in bold letters, the optimal initiation sequence for translation is italicized, and the restriction sites for restriction enzymes are underlined.--

Amendments to the Claims:

Please renumber claim pages 88-94 as pages 104-110.

REMARKS/ARGUMENTS

By the present amendment, Applicant has inserted SEQ ID NOS 8-38 into Sequence Listing. Support for this addition can be found on pages 4, 19, 20 and 47. As requested, Applicant has also inserted SEQ ID NOS: on pages 4, 19, 20 and 47 of the specification. No new matter has been entered by the present amendment and its entry is respectfully requested.

In order to comply with the requirements of 37 C.F.R. 1.821-1.825, Applicants are submitting herewith (1) a Sequence Listing in paper form; (2) a Sequence Listing in computer readable form (a 3.5" floppy diskette) in the ASCII format and (3) a statement (set forth below) that the paper form and the computer readable form of the Sequence Listing are the same.

In accordance with the requirements of 37 C.F.R. 1.821-1.825 the undersigned verifies that the paper form of the Sequence Listing and the computer readable form of the Sequence Listing are the same. No new matter has been added.

Additionally, if further amendments are required to the Sequence Listing in order to complete the requirements for acceptance under 35 U.S.C. 371, Applicants respectfully request that the undersigned be contacted immediately.

Respectfully submitted,

BERESKIN & PARR

By 
Micheline Gravelle
Reg. No. 40,261

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